

16/559434

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(FILE 'HOME' ENTERED AT 09:55:04 ON 27 FEB 2007)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 09:55:18 ON 27 FEB 2007
SEA (HYDANTOIN RACEMASE)

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5 FILE FSTA
139 FILE GENBANK
23 FILE IFIPAT
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16 FILE LIFESCI
17 FILE MEDLINE
16 FILE PASCAL
12 FILE PCTGEN
1 FILE PROMT
25 FILE SCISEARCH
7 FILE TOXCENTER
33 FILE USPATFULL
8 FILE USPAT2
21 FILE WPIDS
21 FILE WPINDEX
L1 QUE (HYDANTOIN RACEMASE)

FILE 'CAPLUS, BIOTECHDS, SCISEARCH, IFIPAT, BIOSIS, WPIDS' ENTERED AT
09:58:53 ON 27 FEB 2007

L2 190 S L1
L3 21 S L2 AND (MUTANT OR VARIANT)
L4 9 DUP REM L3 (12 DUPLICATES REMOVED)

=>

=> d 14 ibib ab 1-9

L4 ANSWER 1 OF 9 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 1

ACCESSION NUMBER: 2006-27154 BIOTECHDS

TITLE: Novel 5-substituted **hydantoin racemase**
polypeptide useful for racemization of optically active
5-substituted hydantoin, and for producing optically active
N-carbamyl amino acid or amino acid;
involving vector plasmid pBHR001-mediated gene transfer
and expression in *Bacillus* sp. or *Escherichia coli*

AUTHOR: NISHI K; YANAGISAWA S; NANBA H; UEDA M; NORO N

PATENT ASSIGNEE: KANEKA CORP

PATENT INFO: WO 2006080409 3 Aug 2006

APPLICATION INFO: WO 2006-JP301253 26 Jan 2006

PRIORITY INFO: JP 2005-22802 31 Jan 2005; JP 2005-22802 31 Jan 2005

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2006-755827 [77]

AB DERWENT ABSTRACT:

NOVELTY - A 5-substituted **hydantoin racemase**
polypeptide (I) having molecular weight of 139000, Km value of 0.304 mM
with respect to L-5-(2-methylthioethyl)hydantoin, range of temperature
being 25-65degreesC (optimum temperature being 40degreesC), range of pH
being 6-10 (optimum pH being 8-9), temperature stability at 30degreesC or
less, and pH stability at 4.5-8.0, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1)
DNA encoding (I) having amino acid sequence of SEQ ID Number 1, consisting
of base sequence of SEQ ID Number 2 or its homologous base sequence; (2)
recombinant plasmid comprising the DNA encoding (I); (3) transformant
obtained by transforming host microorganism with the recombinant plasmid;
(4) microorganism belonging to *Bacillus* genus, capable of producing (I);
(5) producing (I); (6) racemization method of optically active
5-substituted hydantoin, comprises allowing (I), the transformant or the
microorganism having capability of producing (I), to act on an optically
active 5-substituted hydantoin compound; (7) producing optically active
N-carbamyl amino acid, involves allowing hydantoinase and (I), the
transformant or the microorganism having capability of producing (I), to
act on 5-substituted hydantoin compound; and (8) producing optically
active amino acid, comprises allowing hydantoinase, N-carbamyl amino acid
amide hydrolase and (I), the transformant or the microorganism having
capability of producing (I), to act on 5-substituted hydantoin compound.

BIOTECHNOLOGY - Preparation (claimed): (I) is produced by culturing
the microorganism having capability of producing (I), such that (I) is
accumulated in a culture, and extracting (I) from the culture. The
microorganism having capability of producing (I) includes the
transformant or the microorganism of *Bacillus* genus. Preferred
Polypeptide: (I) is obtained from the microorganism belonging to *Bacillus*
genus such as *Bacillus* sp. KNK519HR strain (FERM BP-10477). Preferred
Plasmid: The recombinant plasmid is plasmid pBHR001. Preferred
Transformant: The host microorganism is *Escherichia coli*, and the
transformant is *E. coli* HB101(pBHR001) (FERM BP-10476). Preferred
Microorganism: The microorganism capable of producing (I), is *Bacillus*
sp. KNK519HR strain (FERM BP-10477) or its **mutant**.

USE - For racemization method of optically active 5-substituted
hydantoin, and for manufacturing optically active N-carbamyl amino acid
chosen from N-carbamyl-D-leucine, N-carbamyl-D-isoleucine,
N-carbamyl-D-valine, N-carbamyl-D-norleucine, N-carbamyl-D-norvaline,
N-carbamyl-D-methionine, N-carbamyl-D-cysteine, N-carbamyl-D-
penicillamine, N-carbamyl-D-phenylalanine, N-carbamyl-D-phenyl glycine
and N-carbamyl-D-4-hydroxyphenyl glycine. For producing optically active
amino acid chosen from D-leucine, D-isoleucine, D-valine, D-norleucine,
D-norvaline, D-methionine, D-cysteine, D-penicillamine, D-phenylalanine,

D-phenyl glycine and D-4-hydroxyphenyl glycine (all claimed).

ADVANTAGE - (I) enables efficient production of N-carbamyl amino acid or optically active amino acid.

EXAMPLE - Microorganisms such as *Bacillus* sp. KNK519HR strain (FERM BP-10477) was inoculated to the culture medium comprising glycerol (in weight%) (1), glucose (0.5), potassium dihydrogen phosphate (KH₂PO₄) (0.454), disodium hydrogen phosphate (Na₂HPO₄) (0.620), diammonium sulfate ((NH₄)₂SO₄) (0.65), heptahydrate magnesium sulfate (MgSO₄·7H₂O) (0.05), tetrahydrate manganese chloride (MnCl₂·4H₂O) (0.002), heptahydrate ferrous sulfate (FeSO₄·4H₂O) (0.002), dihydrated calcium chloride (CaCl₂·2H₂O) (0.002), anhydrous sodium citrate (0.032), DL-5-(2-methylthioethyl)hydantoin (0.05), DL-5-methyl hydantoin (0.05) and DL-5-benzyl-hydantoin (0.05), and shaking culture was aerobically was carried out at 30degreesC for 17 hours. To the culture, the sterilized glucose solution was added. After cultivation, the microbial cells were separated by centrifugation, suspended in 50 mM potassium phosphate buffer comprising 1 mM dithiothreitol (DTT), crushed and subjected to centrifugation. After centrifugation, the ammonium sulfate was added to the supernatant liquid (crude-enzyme liquid), and the precipitate was obtained by centrifugation. The precipitate was dissolved in 50 mM potassium phosphate buffer comprising 1 mM DTT, and subjected to dialysis and column chromatography using TSKgel diethylaminoethyl Toyopearl 650 M, to obtain 93% pure **hydantoin racemase**. The isolated **hydantoin racemase** was found to exhibit substrate specificity with respect to D-5-substituted hydantoin compound. (51 pages)

L4 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2006:1305721 CAPLUS

DOCUMENT NUMBER: 146:179326

TITLE: Site-directed mutagenesis indicates an important role of cysteines 76 and 181 in the catalysis of **hydantoin racemase** from *Sinorhizobium meliloti*

AUTHOR(S): Martinez-Rodriguez, Sergio; Andujar-Sanchez, Montserrat; Neira, Jose L.; Clemente-Jimenez, Josefa M.; Jara-Perez, Vicente; Rodriguez-Vico, Felipe; Heras-Vazquez, Francisco J. Las

CORPORATE SOURCE: Departamento Quimica Fisica, Bioquimica y Quimica Inorganica, Universidad de Almeria, Almeria, 04120, Spain

SOURCE: Protein Science (2006), 15(12), 2729-2738

CODEN: PRCIEI; ISSN: 0961-8368

PUBLISHER: Cold Spring Harbor Laboratory Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Hydantoin racemase** enzyme plays a crucial role in the reaction cascade known as "hydantoinase process.". In conjunction with a stereoselective hydantoinase and a stereospecific carbamoylase, it allows the total conversion from D,L-5-monosubstituted hydantoins, with a low rate of racemization, to optically pure D- or L-amino acids. Residues Cys-76 and Cys-181 belonging to **hydantoin racemase** from *Sinorhizobium meliloti* (SmeHyuA) have been proved to be involved in catalysis. Here, we report biophys. data of SmeHyuA Cys-76 and Cys-181 to alanine **mutants**, which point toward a two-base mechanism for the racemization of 5-monosubstituted hydantoins. The secondary and the tertiary structure of the **mutants** were not significantly affected, as shown by CD. Calorimetric and fluorescence expts. have shown that Cys-76 is responsible for recognition and proton retrieval of D-isomers, while Cys-181 is responsible for L-isomer recognition and racemization. This recognition process is further supported by measurements of protein stability followed by chemical denaturation in the presence of the corresponding compound

REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2006:178846 CAPLUS
DOCUMENT NUMBER: 144:407108
TITLE: Binding studies of **hydantoin racemase** from *Sinorhizobium meliloti* by calorimetric and fluorescence analysis
AUTHOR(S): Andujar-Sanchez, Montserrat; Martinez-Rodriguez, Sergio; Las Heras-Vazquez, Francisco Javier; Clemente-Jimenez, Josefa Maria; Rodriguez-Vico, Felipe; Jara-Perez, Vicente
CORPORATE SOURCE: Dpto. Quimica Fisica, Bioquimica y Quimica Inorganica, Universidad de Almeria, Almeria, 04120, Spain
SOURCE: Biochimica et Biophysica Acta, Proteins and Proteomics (2006), 1764(2), 292-298
CODEN: BBAPBW; ISSN: 1570-9639
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Hydantoin racemase** enzyme together with a stereoselective hydantoinase and a stereospecific D-carbamoylase guarantee the total conversion from D,L-5-monosubstituted hydantoins with a low velocity of racemization, to optically pure D-amino acids. **Hydantoin racemase** from *Sinorhizobium meliloti* was expressed in *Escherichia coli*. Calorimetric and fluorescence expts. were then carried out to obtain the thermodyn. binding parameters, ΔG , ΔH and ΔS for the inhibitors L- and D-5-methylthioethyl-hydantoin. The number of active sites is four per enzyme mol. (one per monomer), and the binding of the inhibitor is entropically and enthalpically favored under the exptl. conditions studied. In order to obtain information about amino acids involved in the active site, four different mutants were developed in which cysteines 76 and 181 were mutated to Alanine and Serine. Their behavior shows that these cysteines are essential for enzyme activity, but only cysteine 76 affects the binding to these inhibitors.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2004:1127509 CAPLUS
DOCUMENT NUMBER: 142:70788
TITLE: Screening for improved **hydantoin racemase** mutants, characterization of **hydantoin racemase** mutants from *Arthrobacter crystallopoietes*, and their use in the preparation of amino acids
INVENTOR(S): May, Oliver; Drauz, Karlheinz; Buchholz, Stefan
PATENT ASSIGNEE(S): Degussa Ag, Germany
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004111227	A2	20041223	WO 2004-EP5239	20040515
WO 2004111227	A3	20050616		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ,

TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
 AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
 EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,
 SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
 SN, TD, TG

DE 102004022065 A1 20050317 DE 2004-102004022065 20040505
 US 2006210989 A1 20060921 US 2005-559434 20051205
 PRIORITY APPLN. INFO.: DE 2003-10326109 A 20030606
 DE 2004-102004022065A 20040505
 WO 2004-EP5239 W 20040515

AB The present invention relates to a screening process for **hydantoin racemases** and to novel **hydantoin racemases**, to the nucleic acid sequences coding therefor and to a process for mutagenesis. **Hydantoin racemases** are of interest in connection with the production of enantiomerically enriched amino acids and N-carbamoyl amino acids from racemic hydantoins. More specifically, preparation of **mutant hydantoin racemases** from *Arthrobacter crystallopoietes* with improved enzymic properties is described. The nucleotide sequences and the encoded amino acid sequences of the improved **mutant hydantoin racemases** from *A. crystallopoietes* are disclosed.

L4 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 2000:707276 CAPLUS

DOCUMENT NUMBER: 133:278038

TITLE: Hydantoinase **variants** with improved properties and their use for the production of amino acids

INVENTOR(S): Arnold, Frances H.; May, Oliver; Drauz, Karlheinz; Bommarius, Andreas

PATENT ASSIGNEE(S): California Institute of Technology, USA; Degussa-Huls A.-G.

SOURCE: PCT Int. Appl., 50 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000058449	A1	20001005	WO 2000-US8159	20000328
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6524837	B1	20030225	US 2000-497585	20000203
EP 1165763	A1	20020102	EP 2000-921477	20000328
EP 1165763	B1	20050622		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2003521235	T	20030715	JP 2000-608730	20000328
AT 298365	T	20050715	AT 2000-921477	20000328
EP 1586636	A1	20051019	EP 2004-26624	20000328
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
PT 1165763	T	20051031	PT 2000-921477	20000328
ES 2244430	T3	20051216	ES 2000-921477	20000328
PRIORITY APPLN. INFO.:			US 1999-126923P	P 19990329

US 1999-157427P P 19991004
US 2000-497585 A 20000203
EP 2000-921477 A3 20000328
WO 2000-US8159 W 20000328

AB Hydantoinase enzymes which are **mutants** of Arthrobacter DSM-9771 hydantoinase are disclosed. ~~The mutants include amino acid substitutions at positions 95, 154, 180, 251 and/or 255 of the wild type hydantoinase.~~ The **mutant** hydantoinases, like the parent hydantoinase, are used in the production of optically pure amino acids. Thus, a **mutant** gene encoding Arthrobacter hydantoinase with I95L, V180A, and Q251R substitutions was prepared. This gene, as well as an Arthrobacter carbamoylase gene, was expressed in Escherichia coli. When used to prepare L-Met from D,L-Met hydantoin, the recombinant bacteria produced about 65 mM L-Met per h while the bacteria expressing the wild-type hydantoinase produced only 8 mM per h. The **mutant** enzyme was not enantioselective, but was 4-fold more active than the wild-type enzyme.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 2000:188897 CAPLUS

DOCUMENT NUMBER: 132:278227

TITLE: Inverting enantioselectivity by directed evolution of hydantoinase for improved production of L-methionine
AUTHOR(S): May, Oliver; Nguyen, Peter T.; Arnold, Frances H.
CORPORATE SOURCE: Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, 91125, USA

SOURCE: Nature Biotechnology (2000), 18(3), 317-320
CODEN: NABIF9; ISSN: 1087-0156

PUBLISHER: Nature America

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Using directed evolution, the hydantoinase process for production of L-methionine (L-met) in Escherichia coli was improved. This was accomplished by inverting the enantioselectivity and increasing the total activity of a key enzyme in a whole-cell catalyst. The selectivity of all known hydantoinases for D-5-(2-methylthioethyl)hydantoin (D-MTEH) over the L-enantiomer leads to the accumulation of intermediates and reduced productivity for the L-amino acid. Random mutagenesis, saturation mutagenesis, and screening were used to convert the D-selective hydantoinase from Arthrobacter sp. DSM 9771 into an L-selective enzyme and increased its total activity 5-fold. Whole E. coli cells expressing the evolved L-hydantoinase, an L-N-carbamoylase, and a **hydantoin racemase** produced 91 mM L-met from 100 mM D,L-MTEH in less than 2 h. The improved hydantoinase increased productivity fivefold for >90% conversion of the substrate. The accumulation of the unwanted intermediate D-carbamoyl-methionine was reduced fourfold compared to cells with the wild-type pathway. Highly D-selective hydantoinase **mutants** were also discovered. Enantioselective enzymes rapidly optimized by directed evolution and introduced into multienzyme pathways may lead to improved whole-cell catalysts for efficient production of chiral compds.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2000:327334 CAPLUS

TITLE: Inverting enantioselectivity of a key enzyme creates a viable process for production of L-methionine.

AUTHOR(S): Arnold, Frances H.; Nguyen, Peter T.; May, Oliver

CORPORATE SOURCE: Division of Chemistry and Chemical Engineering, MC 210-41, California Institute of Technology, Pasadena,

SOURCE: CA, 91125, USA
Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-112.
American Chemical Society: Washington, D. C.
CODEN: 69CLAC

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB We have dramatically improved the hydantoinase process for production of L-methionine (L-met) in *E. coli* by inverting the enantioselectivity of a key enzyme using directed evolution. All known hydantoinases are selective for D-5-(2-methylthioethyl)hydantoin (D-MTEH) over the L-enantiomer, which leads to the accumulation of intermediates and reduced productivity for the L-amino acid. Using random mutagenesis, saturation mutagenesis and screening, we converted the D-selective hydantoinase from *Arthrobacter* sp. DSM 9771 (eeD=40% at 30% conversion) into an L-selective enzyme (eeL=20%) and increased its catalytic activity 5-fold. *coli* cells expressing the evolved L-hydantoinase, an L-N-carbamoylase, and a **hydantoin racemase** produced 91 mM L-met from 100 mM D,L-MTEH in less than 2 h. The improved hydantoinase increased productivity 5-fold for > 90% conversion of the substrate. The accumulation of the unwanted intermediate D-carbamoyl-methionine was reduced 4-fold compared to cells with the wild-type pathway. Highly D-selective (<90% eeD at 30% conversion) hydantoinase **mutants** were also discovered. Enantioselective enzymes rapidly optimized by directed evolution and introduced into multi-enzyme pathways lead to improved whole-cell catalysts for efficient production of chiral compds.

L4 ANSWER 8 OF 9 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 6

ACCESSION NUMBER: 1996-12962 BIOTECHDS

TITLE: New microorganism DSM 9771 and use for recovery of gene coded with carbamoylase, hydantoinase or **hydantoin-racemase**;

L-alpha-amino acid e.g. methionine production

AUTHOR: Wagner F; Hantke B; Wagner T; Drauz K; Bommarius A

PATENT ASSIGNEE: Degussa

LOCATION: Frankfurt, Germany.

PATENT INFO: DE 19519717 22 Aug 1996

APPLICATION INFO: DE 1995-119717 30 May 1995

PRIORITY INFO: DE 1995-119717 30 May 1995

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1996-372118 [38]

AB A microorganism, DSM 9771, (or a **mutant**) harboring genes encoding carbamoylase (I), and/or dihydropyrimidinase (EC-3.5.2.2), or **hydantoin-racemase**, is claimed. DSM 9771 (or enzymes from the microorganism) can be used to produce L-alpha-amino acids, by enzymatic conversion of a D-, L-, and/or D,L,N-carbamoyl-alpha-amino acid. The new microorganism has high activity and produces a series of enzymes in large amounts. In an example, a wild strain of DSM 7330 was cultured 10 times on 5 ml of a culture medium containing 10.0 g/l glucose, 0.95 g/l KH₂PO₄, 2.0 g/l K₂HPO₄.3H₂O, 0.2 g/l MgSO₄.7H₂O, 0.02 g/l CaCl₂.2H₂O, 2.0 g/l L-carbamoylmethionine, and 10 ml/l of a solution of trace elements. Single colonies were isolated and incubated on agar plates for 4 days at 30 deg, and then stored in a culture medium containing 10.0 g/l yeast extract, 10.0 g/l bactopectone, 10.0 g/l glucose, 3.0 g/l NaCl, 0.1 g/l MgCl₂.4H₂O, 10 ml/l trace solution, and 15.0 g/l agar. Under N₂, a suspension of 15 g of DSM 9771 and 2.25 g of D,L-methylthioethylhydantoin in 150 ml of saline was incubated at 37 deg and pH 8.5 for 30 hr. Separation yielded 1.6 g (82.6%) of L-methionine. (10pp)

L4 ANSWER 9 OF 9 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 7

ACCESSION NUMBER: 1995-01724 BIOTECHDS

TITLE: New microorganism for L-alpha-amino acid production;
dihydropyrimidinase and hydantion-racemase gene for use in
strain improvement for monosubstituted hydantoin or
carbamoyl-alpha-amino acid conversion

AUTHOR: Wagner F; Voelkel D; Bommarius A; Drauz K

PATENT ASSIGNEE: Degussa

PATENT INFO: EP 625571 23 Nov 1994

APPLICATION INFO: EP 1994-107323-11 May 1994

PRIORITY INFO: DE 1993-4316928 19 May 1993

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 1995-359746 [01]

AB New microorganisms DSM 7329 and DSM 7330 are claimed for use in
L-alpha-amino acid production from D-, L- and/or D,L-5-monosubstituted
hydantoins and/or D,L-N-carbamoyl-alpha-amino acids. Also claimed are
mutants and **variants** of the new microorganisms; use of
the **mutants**, **variants** and isolated enzymes
(dihydropyrimidinase (EC-3.5.2.2) and/or hydantion-racemase) from the
microorganisms for L-amino acid production; and the enzyme encoding genes
from the new microorganisms for use in cell transformation to facilitate
L-amino acid production. (18pp)

Refine Search

Search Results -

Term	Documents
HYDANTOIN	9967
HYDANTOINS	3953
RACEMASE	3575
RACEMASES	372
(HYDANTOIN ADJ RACEMASE).CLM..PGPB,USPT,USOC,EPAB,JPAB,DWPI.	19
((HYDANTOIN RACEMASE).CLM.).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	19

Database:

US Pre-Grant Publication Full-Text Database
 US Patents Full-Text Database
 US OCR Full-Text Database
 EPO Abstracts Database
 JPO Abstracts Database
 Derwent World Patents Index
 IBM Technical Disclosure Bulletins

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Recall Text

Clear

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Search History

DATE: Tuesday, February 27, 2007 [Purge Queries](#) [Printable Copy](#) [Create Case](#)

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<u>L3</u>	(hydantoin racemase).clm.	19	<u>L3</u>
<u>L2</u>	L1 and (position 79)	2	<u>L2</u>
<u>L1</u>	hydantoin racemase	70	<u>L1</u>

END OF SEARCH HISTORY

Hit List

First Hit

Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: US 20060210989 A1

L2: Entry 1 of 2

File: PGPB

Sep 21, 2006

PGPUB-DOCUMENT-NUMBER: 20060210989

PGPUB-FILING-TYPE:

DOCUMENT-IDENTIFIER: US 20060210989 A1

TITLE: Screening process for hydantoin racemases

PUBLICATION-DATE: September 21, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
May; Oliver	Frankfurt		DE
Drauz; Karlheinz	Freigericht		DE
Buchholz; Stefan	Hanau		DE

US-CL-CURRENT: 435/6; 435/106, 435/15, 435/233, 435/325, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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☐ 2. Document ID: US 20060210989 A1, WO 2004111227 A2, DE 1004022065 A1

L2: Entry 2 of 2

File: DWPI

Sep 21, 2006

DERWENT-ACC-NO: 2005-066238

DERWENT-WEEK: 200663

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TITLE: Screening for hydantoin racemases by allowing enantioselective hydantoinase and hydantoin racemase to act on chiral hydantoin and detecting obtained N-carbamoylamino acid or freed protons

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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Term	Documents
POSITION	6741003

POSITIONS	2338275
"79"	900142
79S	213
(1 AND (POSITION ADJ "79")) . PGPB, USPT, USOC, EPAB, JPAB, DWPI.	2
(L1 AND (POSITION 79)) . PGPB, USPT, USOC, EPAB, JPAB, DWPI.	2

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[Generate OACS](#)

Search Results - Record(s) 1 through 19 of 19 returned.

☐ 1. Document ID: US 20060246553 A1

L3: Entry 1 of 19

File: PGPB

Nov 2, 2006

PGPUB-DOCUMENT-NUMBER: 20060246553

PGPUB-FILING-TYPE:

DOCUMENT-IDENTIFIER: US 20060246553 A1

TITLE: 5-substituted hydantoin racemase, DNA coding for the racemase, and processes for producing optically active amino acids

PUBLICATION-DATE: November 2, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Suzuki; Shunichi	Kanagawa		JP
Onishi; Norimasa	Kanagawa		JP
Yokozeiki; Kenzo	Kanagawa		JP

US-CL-CURRENT: [435/108](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 2. Document ID: US 20060210989 A1

L3: Entry 2 of 19

File: PGPB

Sep 21, 2006

PGPUB-DOCUMENT-NUMBER: 20060210989

PGPUB-FILING-TYPE:

DOCUMENT-IDENTIFIER: US 20060210989 A1

TITLE: Screening process for hydantoin racemases

PUBLICATION-DATE: September 21, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
May; Oliver	Frankfurt		DE
Drauz; Karlheinz	Freigericht		DE
Buchholz; Stefan	Hanau		DE

US-CL-CURRENT: [435/6](#); [435/106](#), [435/15](#), [435/233](#), [435/325](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 3. Document ID: US 20060035321 A1

L3: Entry 3 of 19

File: PGPB

Feb 16, 2006

PGPUB-DOCUMENT-NUMBER: 20060035321

PGPUB-FILING-TYPE:

DOCUMENT-IDENTIFIER: US 20060035321 A1

TITLE: Hydantoin racemase

PUBLICATION-DATE: February 16, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Boesten; Wilhelmus Hubertus Joseph	Sittard		NL
Kierkels; Joannes Gerardus Theodorus	Sittard		NL
Assema; Bernard Jan	Geleen		NL
Fuiz Perez; Luis Miguel	Granada		ES
Gonzalez Pacanowska; Dolores	Granada		ES
Gonzalez Lopez; Jesus	Granada		ES
De La Escal Era Hueso; Santiago	Mojaca		ES

US-CL-CURRENT: [435/69.1](#); [435/106](#), [435/233](#), [435/320.1](#), [435/325](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 4. Document ID: US 20050244936 A1

L3: Entry 4 of 19

File: PGPB

Nov 3, 2005

PGPUB-DOCUMENT-NUMBER: 20050244936

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050244936 A1

TITLE: Hydantoin-racemase

PUBLICATION-DATE: November 3, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Altenbuchner, Josef	Nufringen	DE	DE
Mattes, Ralf	Stuttgart	GA	DE
Pietzsch, Markus	Halle		DE
Syldatk, Christoph	Stuttgart		US
Wiese, Anja	Eching		DE
Bommarius, Andreas	Atlanta		US

Tischer, Wilhelm

Peissenberg

DE

US-CL-CURRENT: [435/106](#); [435/233](#), [435/252.3](#), [435/471](#), [536/23.2](#), [536/24.3](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 5. Document ID: US 20050214912 A1

L3: Entry 5 of 19

File: PGPB

Sep 29, 2005

PGPUB-DOCUMENT-NUMBER: 20050214912

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050214912 A1

TITLE: Method for producing an optically active amino acid

PUBLICATION-DATE: September 29, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Nozaki, Hiroyuki	Kawasaki-shi		JP
Watanabe, Kunihiro	Kawasaki-shi		JP

US-CL-CURRENT: [435/106](#); [435/233](#), [435/252.33](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 6. Document ID: US 20050202542 A1

L3: Entry 6 of 19

File: PGPB

Sep 15, 2005

PGPUB-DOCUMENT-NUMBER: 20050202542

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050202542 A1

TITLE: Production method of D-alloisoleucine

PUBLICATION-DATE: September 15, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Maruyama, Shogo	Kawasaki-shi		JP
Kira, Ikuo	Kawasaki-shi		JP
Takemoto, Tadashi	Kawasaki-shi		JP

US-CL-CURRENT: [435/106](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 7. Document ID: US 20050112729 A1

L3: Entry 7 of 19

File: PGPB

May 26, 2005

PGPUB-DOCUMENT-NUMBER: 20050112729
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050112729 A1

TITLE: Recombinant DNA having hydantoinase gene and carbamylase gene and process
for producing amino acid

PUBLICATION-DATE: May 26, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Kira, Ikuo	Kanagawa		JP
Takenaka, Yasuhiro	Kanagawa		JP
Nozaki, Hiroyuki	Kanagawa		JP
Watanabe, Kunihiro	Kanagawa		JP

US-CL-CURRENT: 435/106; 435/228, 435/252.33

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 8. Document ID: US 20050084946 A1

L3: Entry 8 of 19

File: PGPB

Apr 21, 2005

PGPUB-DOCUMENT-NUMBER: 20050084946
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050084946 A1

TITLE: 5-substituted hydantoin racemase, DNA coding for the racemase, and processes
for producing optically active amino acids

PUBLICATION-DATE: April 21, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Suzuki, Shunichi	Kanagawa		JP
Onishi, Norimasa	Kanagawa		JP
Yokozeki, Kenzo	Kanagawa		JP

US-CL-CURRENT: 435/233; 435/252.3, 435/320.1, 435/6, 435/69.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 9. Document ID: US 20030175910 A1

L3: Entry 9 of 19

File: PGPB

Sep 18, 2003

PGPUB-DOCUMENT-NUMBER: 20030175910
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030175910 A1

TITLE: Whole cell catalyst

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Altenbuchner, Josef	Nufringen	GA	DE
Bommarius, Andreas	Atlanta		US
Mattes, Ralf	Stuttgart		DE
Syldatk, Christoph	Stuttgart		DE
Tischer, Wilhelm	Peissenberg		DE
Wiese, Anja	Eching		DE
Wilms, Burkard	Stuttgart		DE

US-CL-CURRENT: 435/106; 435/228, 435/252.33

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 10. Document ID: US 20030166178 A1

L3: Entry 10 of 19

File: PGPB

Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030166178
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030166178 A1

TITLE: Process for the production of enantiomer-enriched alpha-substituted carboxylic acids

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
May, Oliver	Frankfurt		DE
Syldatk, Christoph	Stuttgart		DE
Vielhauer, Oliver	Stuttgart		DE
Werner, Markus	Weinsberg		DE

US-CL-CURRENT: 435/136

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De
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☐ 11. Document ID: US 20030143244 A1

L3: Entry 11 of 19

File: PGPB

Jul 31, 2003

PGPUB-DOCUMENT-NUMBER: 20030143244
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030143244 A1

TITLE: Arthrobacter D-carbamoylase and methods of preparing enantiomerically enriched D-amino acids

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Drauz, Karlheinz	Freigericht	GA	DE
May, Oliver	Frankfurt		DE
Bommarius, Andreas	Atlanta		US
Syldatk, Christoph	Stuttgart		DE
Altenbuchner, Josef	Nufingen		DE
Werner, Markus	Weinsberg		DE
Siemann-Herzberg, Martin	Wildberg		DE

US-CL-CURRENT: [424/190.1](#); [435/196](#), [435/252.3](#), [435/320.1](#), [435/69.3](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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☐ 12. Document ID: US 20020132848 A1

L3: Entry 12 of 19

File: PGPB

Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132848
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132848 A1

TITLE: Process for the preparation of allysine acetal

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Krimmer, Hans-Peter	Dietzenbach		DE
May, Oliver	Frankfurt		DE
Klement, Ingo	Pohlheim-Garbenteich		DE
Drauz, Karlheinz	Freigericht		DE
Reichert, Dietmar	Eschau		DE

US-CL-CURRENT: [514/561](#); [435/106](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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☐ 13. Document ID: US 20020102713 A1

L3: Entry 13 of 19

File: PGPB

Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020102713
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020102713 A1

TITLE: 5-substituted hydantoin racemase, DNA coding for the racemase, and processes for producing optically active amino acids

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Suzuki, Shunichi	Kanagawa		JP
Onishi, Norimasa	Kanagawa		JP
Yokozeki, Kenzo	Kanagawa		JP

US-CL-CURRENT: [435/233](#); [435/106](#), [435/320.1](#), [435/325](#), [435/69.1](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw. De
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☐ 14. Document ID: US 6825014 B2

L3: Entry 14 of 19

File: USPT

Nov 30, 2004

US-PAT-NO: 6825014
DOCUMENT-IDENTIFIER: US 6825014 B2

TITLE: Process for the preparation of allysine acetal

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw. De
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☐ 15. Document ID: US 6815195 B2

L3: Entry 15 of 19

File: USPT

Nov 9, 2004

US-PAT-NO: 6815195
DOCUMENT-IDENTIFIER: US 6815195 B2

TITLE: 5-substituted hydantoin racemase, DNA coding for the racemase, and processes for producing optically active amino acids

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw. De
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☐ 16. Document ID: US 6800464 B2

L3: Entry 16 of 19

File: USPT

Oct 5, 2004

US-PAT-NO: 6800464
DOCUMENT-IDENTIFIER: US 6800464 B2

TITLE: Arthrobacter D-carbamoylase and methods of preparing enantiomerically enriched D-amino acids

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 17. Document ID: US 6713288 B1

L3: Entry 17 of 19

File: USPT

Mar 30, 2004

US-PAT-NO: 6713288

DOCUMENT-IDENTIFIER: US 6713288 B1

**** See image for Certificate of Correction ****

TITLE: Whole cell catalysts

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 18. Document ID: US 5827717 A

L3: Entry 18 of 19

File: USPT

Oct 27, 1998

US-PAT-NO: 5827717

DOCUMENT-IDENTIFIER: US 5827717 A

TITLE: Microorganisms their use and method of producing L-.alpha.-amino acids

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 19. Document ID: US 5516660 A

L3: Entry 19 of 19

File: USPT

May 14, 1996

US-PAT-NO: 5516660

DOCUMENT-IDENTIFIER: US 5516660 A

TITLE: Microorganisms, their use and method of producing L-.alpha.-amino acids

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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Term	Documents
HYDANTOIN	9967
HYDANTOINS	3953
RACEMASE	3575
RACEMASES	372

(HYDANTOIN ADJ RACEMASE) .CLM. .PGPB,USPT,USOC,EPAB,JPAB,DWPI.	19
((HYDANTOIN RACEMASE) .CLM.) .PGPB,USPT,USOC,EPAB,JPAB,DWPI.	19

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0982Y4 RHILQ

All there are
G79 → D
Substitution

Qy	1	MRILVINPNSSSALTESVADAAQQVVATGTIISAINPSRGPVIEGSFDEALATFHLEE	60
		:: :: ::: :: : : : :	
Db	3	VQILVVNPNTTASMTETIGAAARAVAGAWTEIIAVTSSTGPDSEGGYDEALAVPGLLME	62
Qy	61	VERAERENPPDAYVIACFGDPGLDAVKELTDPRPVVGVAEAAIHMSFVAATFSIVSILPR	120
		: : : : :: : : : :: : :	
Db	63	IAAGERRG-AQA AVIACFDDTGLDAARAMANIPVIGICEAALSMA SFIAQRFTVVTTTER	121
Qy	121	VRKHLHELVRQAGATNRLASIKLPNLGVMAFHEDEHAALET LKQAAKEAVQEDGAESIVL	180
		: :: :: :: : : : : :	
Db	122	SRVPVEGLVQRYGMAGR-ARVRAADIPVLALED PASGATGKL RDEIARAVEEDRAEAIVL	180
Qy	181	GCAGMVG FARQLSDELGV PVIDPVEAACRVAESLV ALGYQT SKANSY QKPTEKQY	235
		: : : :: : :	
Db	181	GCAGMADLAHQLOADEFLPVIDGVGA A I K OAEALIALGLSTS KRGAYASPLAKSY	235

http://es/ScoreAccessWeb/GetItem.action?AppId=10559434&seqId=1072174&ItemType=... 2/27/2007

<!--StartFragment-->RESULT 16

ADG73873

ID ADG73873 standard; protein; 238 AA.

XX

AC ADG73873;

XX

DT 11-MAR-2004 (first entry)

XX

DE Agrobacterium radiobacter hydantoin racemase.

XX

KW Hydantoin racemase; enzyme.

XX

OS Agrobacterium tumefaciens.

XX

PN WO2003100050-A1.

XX

PD 04-DEC-2003. ?

XX

PF 23-MAY-2003; 2003WO-NL000386.

XX

PR 23-MAY-2002; 2002NL-01020663.

XX

PA (STAM) DSM NV.

XX

PI Boesten WHJ, Kierkels JGT, Assema FBJ, Ruiz Perez LM;

PI Gonzalez Pacanowska D, Gonzalez Lopez J, De La Escalera Hueso S;

XX

DR WPI; 2004-081908/08.

DR N-PSDB; ADG73872.

XX

PT New polypeptide with hydantoin racemase activity, that does not suffer from substrate inhibition, useful for catalyzing the racemization of substituted D or L hydantoins.

XX

PS Claim 2; SEQ ID NO 2; 27pp; English.

XX

CC The present sequence is the protein sequence of Agrobacterium radiobacter hydantoin racemase, an enzyme that catalyses the racemisation of substituted D or L hydantoins. The A. radiobacter enzyme differs from other hydantoin racemases as it does not exhibit substrate inhibition. The invention provides processes for the racemisation of enantiomerically enriched hydantoin compounds and to processes for the preparation of enantiomerically enriched D- or L-alpha amino acids using the hydantoin racemase. Examples from the invention describe the expression of A. radiobacter hydantoin racemase in Escherichia coli, and use of the over-expressed enzyme in racemisation experiments using D-5-benzylhydantoin, L-5-isopropylhydantoin and L-5-methylmercaptoethylhydantoin substrates.

XX

SQ Sequence 238 AA;

Query Match 40.7%; Score 476.5; DB 8; Length 238;
Best Local Similarity 44.0%; Pred. No. 4.6e-42;
Matches 103; Conservative 39; Mismatches 91; Indels 1; Gaps 1;

Qy 1 MRILVINPNSSSALTESVADAAQQVVATGTIISAINPSRGPVIEGSFDEALATFHLIEE 60

| | : | | | | : : : | | : | : | | | | | | | | | | : : |

Db 1 MHIRLINPNSTASMTAQALDSALRVKQAHTTISATNPLDTPVSIIEGGADEALAVPGMLEE 60

Qy 61 VERAERENPPDAYVIACFGDPLDAVKELTDRPVVGVAEAAIHMSFVAATFSIVSILPR 120

: : | | | | | | | | | | | : | : | : | : | : | : | | | : | |

Db 61 IRKGERLG-VDAYVIACFGDPLGHAAREVARGPVIGICQAGIQVAMTISRFRFSIITTLPR 119

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Qy      121 VRKHLHELVRQAGATNRLASIKLPNLGVMAFHEDEHAALETLKQAAKEAVQEDGAESIVL 180
      : :||  ||      :: || |:  || |||  | : : | :|| ||:|:|
Db      120 SIPIIEDLVDNYGAQRHCRRVRRAINLPVLGLEEDPHAAEAMLIREIEAAKKEDAAEAIL 179

Qy      181 GCAGMVG FARQLSDELGVPVIDPVEAACRVAESLVALGYQTSKANSYQKPTEKQ 234
      |||||      :| :  ||||| | || ::||:|  || ||| |:| | | :
Db      180 GCAGMSALCDRLREATGVPVIDGVTA AVKLAEALVGAGYSTSKVNAYDYPRIKE 233
<!--EndFragment-->
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<!--StartFragment-->RESULT 18

ADF86383

ID ADF86383 standard; protein; 243 AA.

XX

AC ADF86383;

XX

DT 26-FEB-2004 (first entry)

XX

DE Flavobacterium hydantoin racemase amino acid sequence.

XX

KW hydantoin racemase; Flavobacterium;

KW optically active amino acid production; enzyme.

XX

OS Flavobacterium sp.

XX

PN JP2003210176-A.

XX

PD 29-JUL-2003.

XX

PF 22-JAN-2002; 2002JP-00013552.

XX

PR 22-JAN-2002; 2002JP-00013552.

XX

PA (AJIN) AJINOMOTO KK.

XX

DR WPI; 2003-819831/77.

DR N-PSDB; ADF86382, ADF86392.

XX

PT Novel protein having penta substituted hydantoin racemase, activity,

PT useful for manufacturing optically active amino acid.

XX

PS Claim 1; SEQ ID NO 2; 30pp; Japanese.

XX

CC This invention relates to a novel protein having penta substituted
 CC hydantoin racemase activity (derived from Flavobacterium) which comprises
 CC a fully defined sequence of 243 amino acids as given in the
 CC specification, optionally containing deletion, insertion, addition or
 CC inversion of one or more amino acid residues. The invention is useful in
 CC the production of optically active amino acids.

XX

SQ Sequence 243 AA;

Query Match 40.6%; Score 474.5; DB 7; Length 243;

Best Local Similarity 41.7%; Pred. No. 7.8e-42;

Matches 98; Conservative 45; Mismatches 91; Indels 1; Gaps 1;

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Qy      1 MRILVINPNSSSALTESVADAAQQVVATGTIISAINPSRGPVIEGSFDEALATFHLIEE 60
      |:| ||||:: :| : :|| | : || : |::|: ||| ||| :||:: :::|
Db      1 MKIKVINPNTTLMTAKIGEAAAASAGTEVVAVSPAMGPASIEGHYDEAVSALGVLDE 60

Qy     61 VERAERENPPDAYVIACFGDPLDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVSILPR 120
      | : : | | |::||| ||| | : : | |||:||||:|:|::| | :|
Db     61 VRKGKAEG-CDGYLIACFDDPGLQAAREIADGPVVGIAEAAAMHMASFVSEGFVSVATGHR 119

Qy    121 VRKHLHELVRQAGATNRLASIKLPNLGVMAFHEDEHAALETLKQAAKEAVQEDGAESIVL 180
      | | | | | : : : | | : : | : : : | : | : | : ||
Db    120 SRIILEHLARSYGMEHKCRKVRTTELAVLDLEVEGSDARGIILEECRAIVEDHSDCIVL 179

Qy    181 GCAGMVGAFARQLSDELGVFVIDPVEAACRVAESLVALGYQTSKANSYQKPTEKQY 235
      |||| | :| |||||:| | | :| | |:| ||:| | | | |
Db    180 GCAGMADLADYISKELGVFVVDGVAAGVKVLEGLIGLRLSTSRACGYAYPNPKTY 234

```


<!--EndFragment-->

<!--StartFragment-->RESULT 16

ADG73873

ID ADG73873 standard; protein; 238 AA.

XX

AC ADG73873;

XX

DT 11-MAR-2004 (first entry)

XX

DE Agrobacterium radiobacter hydantoin racemase.

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PN WO2003100050-A1.

XX

PD 04-DEC-2003.

XX

PF 23-MAY-2003; 2003WO-NL000386.

XX

PR 23-MAY-2002; 2002NL-01020663.

XX

PA (STAM) DSM NV.

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PI Boesten WHJ, Kierkels JGT, Assema FBJ, Ruiz Perez LM;

PI Gonzalez Pacanowska D, Gonzalez Lopez J, De La Escalera Hueso S;

XX

DR WPI; 2004-081908/08.

DR N-PSDB; ADG73872.

XX

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XX

PS Claim 2; SEQ ID NO 2; 27pp; English.

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XX

SQ Sequence 238 AA;

Query Match 40.7%; Score 476.5; DB 8; Length 238;
Best Local Similarity 44.0%; Pred. No. 4.6e-42;
Matches 103; Conservative 39; Mismatches 91; Indels 1; Gaps 1;

Qy 1 MRILVINPNSSSALTESVADAAQQVVATGTIIISAINPSRGPVIEGSFDEALATFHLIEE 60
| | :| | | | : : : | | : | : | | | | | | | | : : | |

Db 1 MHIRLINPNSTASMTAQALDSALRVKQAHTTISATNPLDTPVSIEGGADEALAVPGMLEE 60

Qy 61 VERAERENPPDAYVIACFGDPLDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVSILPR 120
: : | | | | | | | | | | | : | : | : | : | : | : | | | : | |

Db 61 IRKGERLG-VDAYVIACFDDPGLHAAREVARGPVIGICQAGIQVAMTISRFRFSIITTLPR 119

```

Qy      121 VRKHLHELVRQAGATNRLASIKLPNLGVMAFHEDEHAALETQAAKEAVQEDGAESIVL 180
      : :||  ||      :: || |:  || |||  | : : | :|| ||:|:|
Db      120 SIPIIEDLVDNYGAQRHCRRVRRAINLPVLGLEEDPHAAEAMLIREIEAAKKEDAAEAIL 179

Qy      181 GCAGMVG FARQLSDELGVPVIDPVEAACRVAESLVALGYQTSKANSYQKPTEKQ 234
      |||||      :| : ||||| | || ::||:| | || ||| |:| | |:
Db      180 GCAGMSALCDRLREATGVPVIDGVTA AVKLAELVGAGYSTSKVNAYDYPRIKE 233

```

<!--EndFragment-->

<!--StartFragment-->RESULT 9

B71099

probable hydantoin racemase - *Pyrococcus horikoshii*C;Species: *Pyrococcus horikoshii*

C;Date: 14-Aug-1998 #sequence_revision 14-Aug-1998 #text_change 09-Jul-2004

C;Accession: B71099

R;Kawarabayasi, Y.; Sawada, M.; Horikawa, H.; Haikawa, Y.; Hino, Y.; Yamamoto, S.; Sek DNA Res. 5, 55-76, 1998

A;Title: Complete sequence and gene organization of the genome of a hyper-thermophilic

A;Reference number: A71000; MUID:98344137; PMID:9679194

A;Accession: B71099

A;Status: preliminary; nucleic acid sequence not shown; translation not shown

A;Molecule type: DNA

A;Residues: 1-228 <KAW>

A;Cross-references: UNIPROT:O58781; UNIPARC:UPI0000066833; GB:AP000004; NID:g3236131;

A;Experimental source: strain OT3

A;Note: this accession replaces an interim accession for a sequence replaced by GenBan

C;Genetics:

A;Gene: PH1054

Query Match 14.9%; Score 174; DB 2; Length 228;

Best Local Similarity 28.6%; Pred. No. 1.7e-06;

Matches 58; Conservative 29; Mismatches 64; Indels 52; Gaps 8;

```

Qy      43 VIEGSFDEALATFHLIEEV-----ERAERENPP-----DAYVIACFGDPG 82
      :|| :| |      ||:      | ||| |      || :|:| ||
Db      29 IIESAFPELKVVSRICEDQPKGIYNEETEREAEPKIIRLAKEFEREGVDIIISCAADPA 88

Qy      83 LDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVSILPRVRKHLHELVRQAGATNRLASIK 142
      :: |:| | ||:| | :|: | | :| : : ::| | :|
Db      89 VEKVRKLLSIPVIG---AGSSVSALALAYGRRVGVL-NLTEETPKVIRSILGNNLIA--- 141

Qy     143 LPNLGVMAFHEDEHAALET-----LKQAAKEAVQEDGAESIVLGCAGM--VGF 188
      || : :      : ||| :| | | ||| || :|
Db     142 -----EDHPSGVSNTDLLTDWGRREVINAAGR-LKEKGVEVIALGCTGMSTIGI 190

Qy     189 ARQLSDELGVPVIDPVEAACRVA 211
      | | :|:|:| |||| | : ||
Db     191 APVLEEEVGIPVIDPVIASGAVA 213

```

<!--EndFragment-->

<!--StartFragment-->RESULT 8

T41412

probable hydantoin racemase - fission yeast (*Schizosaccharomyces pombe*)C;Species: ~~Schizosaccharomyces pombe~~

C;Date: 03-Dec-1999 #sequence_revision 03-Dec-1999 #text_change 09-Jul-2004

C;Accession: T41412

R;Wood, V.; Rajandream, M.A.; Barrell, B.G.; Murphy, L.; Harris, D.

submitted to the EMBL Data Library, September 1998

A;Reference number: Z21954

A;Accession: T41412

A;Status: preliminary; translated from GB/EMBL/DDBJ

A;Molecule type: DNA

A;Residues: 1-236 <WOO>

A;Cross-references: UNIPROT:O74886; UNIPARC:UPI000006AF98; EMBL:AL031798; PIDN:CAA2118

A;Experimental source: strain 972h-; cosmid c576

C;Genetics:

A;Gene: SPDB:SPCC576.02

A;Map position: 3

Query Match 17.2%; Score 201.5; DB 2; Length 236;

Best Local Similarity 29.5%; Pred. No. 1.2e-08;

Matches 69; Conservative 40; Mismatches 118; Indels 7; Gaps 6;

```

Qy      3 ILVINPNSSSALTESVADAAQQVVATGTIISAIN-PSRGPVIEGSFDEALATFHLIEEV 61
          |||||: :| |: :   :| :   : | | ||: | || | |: :
Db      4 ILVINPNSSTFITTSMEELVPLVPSDVKLRLFTCPQPGAVID-SITEATLTAALVFQA 62

Qy     62 ERAERENPPDAYVIACFG-DPGLDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVSILPR 120
          : || :||: | :| ::| | :| :|:: : | |:: |
Db     63 LTPSVLDGVDAIAVACYSPTPLVDMIRESFALPCMGIVQASVLSALSVMQVRIGILTSTYR 122

Qy    121 VRKHLHELVRQAGAT-NRLASIKLPNLGVMAFHEDEHAALET-LKQAAKEAVQEDGAESI 178
          |:|: | : |:|: | : :   || | | |: | | : |
Db    123 SECLLYELDSFGVSRTRVAAIASTGRTVLQLSQMPSQERETLLVQKAQELANTKGADVI 182

Qy    179 VLGCAGMVGFRQLSDELG--VPVIDPVEAACRVAESLVALGYQTSKANSYQKP 230
          || | : | : :| :|:| | || : | | | | |
Db    183 CLGGAALAAIRDQIQVAVGPNIPIDGVHAAVELLAGLARQNLHTSKFGIYTYP 236

```

<!--EndFragment-->

<!--StartFragment-->RESULT 5

AD3135

hydantoin racemase [imported] - Agrobacterium tumefaciens (strain C58, Dupont)

C;Species: Agrobacterium tumefaciens

C;Date: 11-Jan-2002 #sequence_revision 11-Jan-2002 #text_change 09-Jul-2004

C;Accession: AD3135

R;Wood, D.W.; Setubal, J.C.; Kaul, R.; Monks, D.; Chen, L.; Wood, G.E.; Chen, Y.; Woo, Science 294, 2317-2323, 2001

A;Authors: Yoo, H.; Tao, Y.; Biddle, P.; Jung, M.; Krespan, W.; Perry, M.; Gordon-Kamm

A;Title: The Genome of the Natural Genetic Engineer Agrobacterium tumefaciens C58.

A;Reference number: AB2577; MUID:21608550; PMID:11743193

A;Accession: AD3135

A;Status: preliminary

A;Molecule type: DNA

A;Residues: 1-240 <KUR>

A;Cross-references: UNIPROT:Q8U6V2; UNIPARC:UPI0000164993; GB:AE008689; PIDN:AAL45498.

A;Experimental source: strain C58 (Dupont)

C;Genetics:

A;Gene: huy

A;Map position: linear chromosome

Query Match 39.1%; Score 457.5; DB 2; Length 240;

Best Local Similarity 42.1%; Pred. No. 9.1e-29;

Matches 98; Conservative 41; Mismatches 93; Indels 1; Gaps 1;

```

Qy      1 MRILVINPNSSSALTESVADAAQQVVATGTIISAINPSRGPVIEGSFDEALATFHLIEE 60
        | | :|||||:::| | :| | :|| | | | | | | | | | : |
Db      1 MHIRLINPNSTASMTAQALDSALRVKQKDTHTVSAANPVDTPVSIEGQADEAMAVPGLLAE 60

Qy      61 VERAERENPPDAYVIACFGDPGLDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVSILPR 120
        : : | | | | | | | | | | | : | : | : | : | : | : | : | : | : |
Db      61 IRKGEHG-VDAYVIACFDDPGLHAAREVARGPVIGICQAAVQVAMTISRFRSIITTLPR 119

Qy      121 VRKHLHELVRQAGATNRLASIKLPNLGVMAFHEDEHAALETLKQAAKEAVQEDGAESIVL 180
        : : || | | : : : | | : | | | | : : | : | | | : | : |
Db      120 SIPIIEDLVEDYGAQRYCRKVRAIDLPLVGLLEEDPEVAEALLRREIEAAKREDAEAIIL 179

Qy      181 GCAGMVG FARQLSDELGVPVIDPVEAACRVAESLVALGYQTSKANSYQKPTEK 233
        |||| | : | | | | | | | | | : | : | | | | | | | | | |
Db      180 GCAGMSSLCDRLRDATGVPVIDGVTAIAIKLAEALVGAGYTTSKVNAYDYPRVK 232

```

<!--EndFragment-->

<!--StartFragment-->RESULT 7

S62582

probable hydantoin racemase - fission yeast (*Schizosaccharomyces pombe*)

C;Species: *Schizosaccharomyces pombe*

C;Date: 16-May-1996 #sequence_revision 13-Mar-1997 #text_change 09-Jul-2004

C;Accession: S62582; T38104

R;Murphy, L.; Niblett, D.; Harris, D.

submitted to the EMBL Data Library, November 1995

A;Reference number: S62573

A;Accession: S62582

A;Status: preliminary

A;Molecule type: DNA

A;Residues: 1-238 <MUR>

A;Cross-references: UNIPROT:Q09921; UNIPARC:UPI000013A105; EMBL:Z67998; NID:g1067202;

R;Beck, A.; Reinhardt, R.; Murphy, L.; Niblett, D.; Harris, D.; Barrell, B.G.; Rajandr submitted to the EMBL Data Library, November 1995

A;Reference number: Z21769

A;Accession: T38104

A;Status: preliminary; translated from GB/EMBL/DDBJ

A;Molecule type: DNA

A;Residues: 1-238 <MU2>

A;Cross-references: UNIPARC:UPI000013A105; EMBL:Z67998; NID:g1067202; PIDN:CAA91957.1;

A;Experimental source: strain 972h-; cosmid c1F7

C;Genetics:

A;Gene: SPAC1F7.10

A;Map position: 1R

Query Match 20.4%; Score 239; DB 2; Length 238;
Best Local Similarity 30.1%; Pred. No. 1.4e-11;
Matches 71; Conservative 42; Mismatches 111; Indels 12; Gaps 7;

```
Qy      1 MRILVINPNSSSALTESVADAAQQVVATGTIISAIN-PSRGPVIEGSFDEALATFHLIE 59
          :|||||||: :|||      : : | || || | : |::
Db      2 IRILVINPNSTVQMTESVKSVLDDCTPPNVQLEYLTCPEGPKAIECVSDGVRSAAVLMK 61

Qy      60 EVERAERENPP--DAYVIACFGD-PGLDAVKELTDRPVVGVAEAAIHMSSFVAATFSIVS 116
          | :|| ||:::|: | | : :| :| :|:| : : |::
Db      62 YFE---DHPPQVDAFLVSCYSDHPLVTTLRETYRKPCGTGIMQASILTALSLGRKVSVVT 117

Qy      117 ILPRVRKHLHELVRQAGATNRL-ASIKLPNLGVMAFHEDEHAALET-LKQAAKEAVQEDG 174
          | | : : | : : | | | : | : : | : | |||
Db      118 TTKRYEPLLTGDIHAMGISDSVFAGIASTGLAPLELDSKPRAEVDALLARTALRAVNEMG 177

Qy      175 AESIVLGCAGMVG FARQLSDELG--VPVIDPVEAACRVAESLVALGYQTSKANSYQ 228
          | : | ||||| | | :| :|:| :| : | | : ||| ||
Db      178 ADVICLGCAGMTHMAHVLEKAVGPNIPIDGKTAGVELLASLVRMNLFTSKQGVYQ 233
```

<!--EndFragment-->